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with increasing Mg content. The Young's modulus of PLGA/TCP/Mg scaffolds (Mg content: 15% wt) was around 104 Mpa, statistically significantly stronger than that of Mg content: 10% wt (83Mpa) and Mg content 5% wt group (82 Mpa), as well as PLGA/Mg group, 66Mpa. All the Mg containing scaffolds were statistically significantly stronger than PLGA/TCP and PLGA group, 45 Mpa and 30 Mpa, respectively.
Conclusion: The results of CCK-8 assay demonstrated the MC3T3-E1 osteoblasts grew very well and proliferated rapidly on PLGA/TCP/Mg scaffolds compared to PLGA/TCP scaffold after 7 day culture. The in vitro study also demonstrated a good biocompatibility and bioactivity of the PLGA/TCP/Mg scaffold that was in favor of accelerating and inducing the proliferation and differentiation of osteoblasts.
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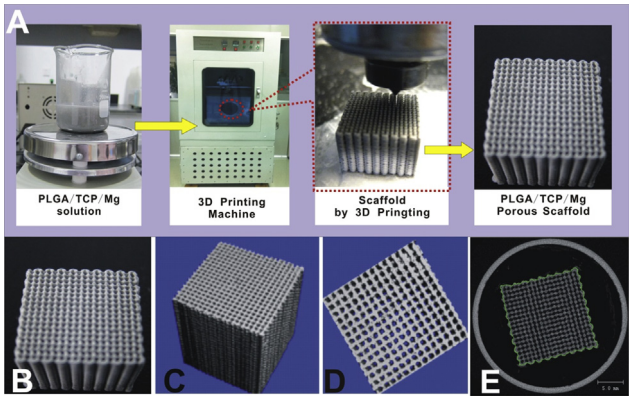

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SYNERGISTIC EFFECT OF DECELLULARIZED ANNULUS FIBROSUS (AF) MATRIX AND SUBSTRATE STIFFNESS ON THE GENE EXPRESSION OF RABBIT AF STEM CELLS

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Objective: Decellularized matrices (DCM) have been widely used for engineering functional tissues, mainly due to the similarity of biochemical composition and microstructure between them and native extracellular matrix (ECM) in vivo. Meanwhile, the mechanical properties of substrate play an important role in regulating cell behavior such as adhesion, proliferation, differentiation and migration. Here, we aimed to study the combined effect of DCM and substrate stiffness on the behaviors of newly identified rabbit AF-derived stem cells (AFSCs).
Methods: To this end, decellularized porcine annulus fibrosus matrix (DAFM) was covalently coupled to polyacrylamide gels (PAGs) which had elastic moduli of 2.6 KPa (Soft), 10.6 KPa (Middle), and 34.9 KPa (Rigid), respectively. As control, collagen-coated PAGs of similar stiffness were used.
Results: After 7 days of culture on these PAGs, AFSCs on soft collagen-coated PAGs exhibited the least expression of collagen-I gene, while cells on rigid collagen-coated PAGs exhibited the greatest. In contrast, cells on soft collagen-coated PAGs exhibited the greatest expression of collagen-II and aggrecan genes, while cells on rigid collagen-coated PAGs had the least expression of them.
Conclusion: The gene expression of cells on middle PAGs was between those on soft and rigid PAGs. Expression of the above genes in AFSCs cultured on DAFM-coated PAGs followed similar substrate stiffness-dependent pattern. However, the responses of AFSCs to substrate stiffness appeared to be more prominent when they were cultured on DAFM-coated PAGs. Therefore, combined use of DAFM and scaffolds of gradient stiffness may provide a more efficient approach for AF tissue engineering.

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ABNORMAL FUNCTIONAL RESPONSES OF OSTEOBLASTS TO LEPTIN IN ADOLESCENT IDIOPATHIC SCOLIOSIS

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Objective: Leptin has been postulated as one of the etiologic factors of AIS because of its important physiological functions in neuro-osseous development affecting skeletal growth, the onset of puberty, energy expenditure and body composition. Previous studies on the relationship between leptin and HR-pQCT derived bone quality parameters had found abnormal correlations in AIS girls, and suggested possible abnormalities in the leptin regulated bone metabolic pathways. Another study on AIS patients showed hyposensitivity to leptin in bone marrow derived mesenchymal stem cells. This study aimed to investigate the effect of leptin on the functional responses of osteoblasts in AIS girls, and compare with that in control subjects.
Material and Methods: In vitro assays were performed with osteoblasts isolated from 12 severe AIS girls and 6 control subjects. The osteoblasts were exposed to different concentrations of leptin (0, 10, 100, 1000 ng/ml). The effects of leptin on cell proliferation were evaluated with MTT assay after 3 days of leptin treatment; differentiation with ALP activity assay after 6 and 14 days, and with osteocalcin ELISA throughout the 35 days of culture; and mineralization with von Kossa staining after 21 and 35 days.
Results: Baseline comparison between osteoblasts from AIS and control groups showed lower differentiation and mineralization potentials in the AIS group. For functional responses to leptin, control group showed increasing proliferative response to leptin in a dose dependent manner ($p=0.008$), while AIS group showed no proliferate response to leptin ($p=0.962$). For differentiation, control group showed strong and significant trend in ALP activity to increasing leptin concentrations in both day 6 ($p=0.012$) and 14 ($p=0.017$), and secreted osteocalcin in an increasing dose dependent manner to leptin ($p=0.007$ in day 35), but this trend were not observed in the AIS group ($p>0.05$). For mineralization, the control group showed a mild rising trend to increasing leptin concentrations ($p=002$), and again no trend was observed in the AIS group ($p=0.305$).
Conclusion: The results in this study suggested that the osteoblasts isolated from AIS girls had low differentiation and mineralization potentials, as well as abnormally low functional responses to leptin when compared with controls. These decreases in functional responses might be due to dysfunction of leptin signaling pathway, which could include abnormalities in the leptin receptor or downstream signal molecules. This is an important finding and might serve to explain the low bone mass and deranged bone quality that is associated with AIS.

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BONY SPUR FORMATION AND DISCUSSION IN COLLAGEN-INDUCED ARTHRITIS RAT MODEL

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Introduction: A systemic chronic joint inflammation leads to profound changes in the joint architecture, which is the structural basis for progressive impairment of function. Radiographic features of RA are those of joint inflammation, periarticular osteopenia, uniform joint space loss, bone erosions, and soft-tissue swelling. Conversely, inflammatory joint destruction is sometimes accompanied by modeling of bony spurs, also termed osteophytes, which emerge at the joint margins in diseases, such as psoriatic arthritis and ankylosing spondylitis. The reason for the apparently divergent bone responses among various inflammatory diseases has not been fully clarified, but appears to involve differential regulation of local bone homeostasis in the course of joint inflammation. With the destruction of joint

cartilage in the late stage, bony spur forms in RA. These symptoms are very important in RA pathological development.
Methods: In this study, CIA was used as an animal model to elucidate further the pathological process of bony spur. The destruction of joints in the CIA model was observed by radiology and histology.
Results: In the radiological observation, bony spur formed in the knee and foot joint, which worsened as the disease progressed. Meanwhile, fusion and damage of articular cartilage was observed, and many osteoclasts were found in the histological sections.
Conclusion: Based on previous research on the CIA model and related investigations, the bony spur may have another main pathological process in the later stages of RA.

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miRNA EXPRESSION PROFILES DURING ADIPOGENIC AND OSTEOGENIC DIFFERENTIATION OF MOUSE BMSCs

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Objective: Identification of miRNA expression profiles during adipogenic and osteogenic differentiation of mouse bone marrow mesenchymal stem cells (BMSCs).
Materials and methods: BMSCs were isolated from the femurs and tibias of 4- to 6-week-old male C57BL/6 mice as previously described, and cultured in α-MEM supplemented with 10% FBS. Then BMSCs were identified by in vitro multi-lineage differentiation assays, including adipogenesis, osteogenesis and chondrogenesis. Subsequently, the cells were cultured in adipogenesis differentiation medium for 6 days and in osteogenesis differentiation medium for 10 days, respectively. Uninduced cells were included as control. miRNA profiles were analyzed using Agilent Mouse miRNA microarray slide (8 X 60K, Part number G4872A). Hierarchical clustering was performed with Multi-experiment Viewer (MeV) software. A selected subset of miRNAs changed more than 1.8-fold was selected for further real-time PCR analysis.
Results: The miRNA microarray analysis showed that 66 miRNAs were differentially expressed during adipogenic or osteogenic differentiation of mouse BMSCs. Real-time PCR analysis showed that, compared with the control, the expression level of miR-218-5p was increased 10-fold and 2.8-fold after adipogenic induction for 6 days and osteogenic induction for 10 days, respectively. Within the first three days of induction, miR-218-5p was increased 5.9-fold during adipogenic differentiation, yet without significant difference during osteogenic differentiation. The expression levels of miR-146a-5p and miR-223-3p were decreased 10.8-fold and 6.6-fold, respectively, during osteogenic differentiation.
Conclusions: miR-218-5p was increased during both adipogenic and osteogenic differentiation, with a significant predominance in adipogenic differentiation. In addition, miR-146a-5p and miR-223-3p were decreased during osteogenic differentiation. An effort will be made to understand their roles and mechanisms.
Acknowledgements
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ABNORMAL BONE MICRO-ARCHITECTURE AND ROD-PLATE CONFIGURATION IN OSTEOPENIC ADOLESCENT IDIOPATHIC SCOLIOSIS (AIS)

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Objectives: Multiple studies have documented the presence of systemic osteopenia in AIS. Osteopenia was associated with severe curves and was reported to be one of the significant prognostic factors for curve progression in AIS. This study aimed to evaluate bone quality and bone strength parameters including rod-plate configuration and finite element analysis (FEA) with in vivo High-Resolution Peripheral Quantitative Computed Tomography (HR-pQCT) and to investigate their relationship with osteopenia in AIS Vs normal controls.
Material and Methods: 101AIS and 105 controls between 11-14 years old were recruited. Areal bone mineral density (aBMD) of bilateral femoral necks was measured with Dual Energy X-ray Absorptiometry (DXA). Subjects were classified into the osteopenic (Z-score$<$or$=$-1) and non-osteopenic (Z-score$>$-1) group. Bone Morphometry, volumetric bone mineral density (vBMD) and Trabecular Bone Micro-architecture were measured using HR-pQCT. Structural Model Index (SMI) quantifying the trabecular rod/plate configuration (a higher index indicating more rod-like configuration) and FEA in terms of Stiffness, Failure Load and Apparent Modulus were calculated with a standard algorithm.
Results: In the AIS group, osteopenic subjects showed higher SMI, lower Stiffness, lower Failure Load and lower Apparent Modulus when compared with non-osteopenic subjects (% difference $=$ 15.5%, -24.5%, -23.1% & -20.5% respectively, all with $p<0.001$). Similar differences in FEA profiles were noted between osteopenic and non-osteopenic subjects in the control group. In contrast, no significant difference in SMI was found between osteopenic and non-osteopenic controls. When all osteopenic subjects were considered, osteopenic AIS subjects had higher SMI when compared with osteopenic controls (% difference $=$ 9.1%, $p=0.012$).
Conclusions: This study showed that osteopenia was associated with lower bone strength and a specific pattern of SMI indicating preponderance of rod-like configuration in AIS subjects. Notably the association of higher SMI with osteopenia was seen in AIS but not in normal controls, thus providing strong evidences that osteopenia in AIS was different from osteopenia in non-AIS controls. Further investigations exploring the underlying biochemical and biomechanical mechanisms that bring about these specific endophenotypes are warranted for gaining further understanding of the etiopathogenesis of AIS.
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EVALUATING BONE STRENGTH WITH FINITE ELEMENT ANALYSIS FOR ADOLESCENT IDIOPATHIC SCOLIOSIS (AIS): A CASE-CONTROL STUDY WITH HR-pQCT

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Objectives: Although Adolescent Idiopathic Scoliosis (AIS) was associated with low bone mass, reports on bone mechanical properties in AIS are sparse. The objective of this study is to evaluate bone mechanical properties with finite element analysis (FEA) using in-vivo High-Resolution Peripheral Quantitative Computed Tomography (HR-pQCT) in AIS and compare that with normal controls.
Material and Methods: 97 AIS girls and 99 female controls between 11-14 years old were recruited. Dietary calcium intake and physical activity level were assessed with a standard Food Frequency Questionnaire and the Modified Baecke Questionnaire respectively. With HR-pQCT, an established model on morphology and micro-structure of the non-dominant distal radius was generated for FEA in terms of Stiffness, Failure Load and Apparent Modulus. Multivariate linear regression analysis was used to investigate the difference between AIS and controls after adjusting for age in Model 1 and for age, calcium intake and physical activity level in Model 2.
Results: 2-tailed Student's t-test showed AIS subjects had lower Stiffness, lower Failure Load and lower Apparent Modulus when compared with normal controls (% difference $=$ -6.81%, -7.10% & -8.10% respectively, all with $p<0.05$). AIS girls had lower Failure Load ($B=-136.0$, $p=0.04$) and lower Apparent Modulus ($B=-146.2$, $p=0.021$) in Model 1 with adjustment for age. In Model 2, difference in Apparent Modulus remained statistically significant with AIS being associated with lower Apparent Modulus after adjustment for age, calcium intake and physical activity level ($B=-137.1$, $p=0.037$).